31. (New) A recombinant DNA according to claim 30, wherein said sequence (1) codes for an antigenic polypeptide or peptide of a pathogenic agent.

C2 corel.

32. (New) A vaccine composition for stimulating mucosal immunity comprising a cell culture according to claim 22 or a recombinant protein encoded by the recombinant DNA of claim 1.

33. (New) A method of stimulating mucosal immunity, comprising nasal administration to a subject in need thereof of a composition comprising a cell culture according to claim 22 or a recombinant protein encoded by the recombinant DNA of claim 1.--

REMARKS

By the present amendment, claim 26 has been deleted without prejudice or disclaimer. In particular, Applicants reserve their rights to file divisional and/or continuation application(s) directed at any canceled subject matter. Claims 1-15, 17, 18, 20, 22, 27 and 28 have been amended to clarify the invention. New claims 30-33 have been added, related to particular embodiments of the present invention. The amendments and newly presented claims are supported by the specification and it is believed that no new matter has been introduced. In this respect, the amendment to claim 8 is supported by the specification, at least page 6 line 22 and page 7 line 31.

Turning now to the Office Action of January 5, 1999, claims 1-22 and 26-29 have been rejected under Article 35 USC 112, second paragraph, for lack of clarity. It is submitted that this rejection has been obviated by the present amendment. Indeed, by the present amendment, the word "*containing*" in claim 1 has been replaced with --*comprising*-- ; the expression "*corresponding essentially to*" in claim 5 has been replaced with --*consisting essentially of*-- and claim 26 has been deleted. It is therefore submitted that the rejection has been overcome and withdrawal of the same is respectfully requested.

Claims 1-5, 7, 10-22 and 26-29 have been rejected under 35 USC 103 as being unpatentable over Menozzi et al. The rejection is respectfully traversed.

In rendering the rejection, the Examiner indicates that Menozzi et al mentions a fusion protein comprised of the amino-terminal domain of mature FHA fused to MalE, which is able to bind heparin. As will be discussed below, there are several important differences between the claimed invention and the teaching of Menozzi et al., and there was no suggestion or expectation of success in the prior art.

(a) The protein of Menozzi et al is intracytoplasmic

As indicated in Menozzi et al, the fusion protein is recovered only after lysis of the cells ("*the cells were centrifuged and resuspended in 25 ml of lysis buffer*", see page 771, left column, for instance). This results from the constructs and plasmids used by Menozzi, which do not allow excretion (or exposure) of the protein outside of the cytoplasm.

To the contrary, the instant invention provides recombinant nucleic acids which (i) encode chimeric proteins comprising a FHA and a heterologous moiety and which (ii) allow secretion and/or exposure of said chimeric proteins outside of the cell cytoplasm. In this respect, claim 1 provides that the protein is "*excreted into the culture medium of these cells or exposed at the surface of these cells*" (emphasis added) and claim 30 provides that the recombinant polypeptide is "*secreted into the culture medium or exposed at the cell surface*".

This is an important distinction between the instantly claimed invention and the teaching of Menozzi et al. As will be discussed below, Menozzi et al. does not suugest at all the secretion of any recombinant polypeptide fused to FHA.

(b) the MalE moiety has no biological activity

The fusion reported in Menozzi comprises a FHA portion and the MalE protein. This construct is made solely for practical reasons. Indeed, MalE is known to bind amylose and has been widely used in affinity isolation methods. In this regard, Menozzi indicates that the fusion with maltose-binding protein (MalE) was made in order to allow "*a rapid and easy affinity purification of recombinant polypeptides, using an amylose-based chromatography*" (see page 774, left column, last paragraph). However, the MalE protein does not exhibit any biological activity and there is no indication in Menozzi that any molecule fused to Fha would retain any biological activity.

In contrast, the invention shows that biologically active polypeptides can be fused to portions of FHA, that the resulting fusion molecule can be secreted or exposed at the cell surface, and that the biologically active polypeptide is functional both in vitro and in vivo. There was no suggestion in Menozzi et al. to arrive to the invention (i.e., prepare the instantly claimed constructs) and no reasonable expectation that they would exhibit such advantageous properties "high immunogenic activity, mucosal immune response, nasal administration, etc.).

In fact, Menozzi et al reports a fundamental study of the binding properties of FHA. For practical purposes only (e.g., facilitate purification), some fusion constructs are mentioned between a portion of FHA and the MalE protein. However, as explained above, these fusions are (i) intracytoplasmic, (ii) the MalE moiety has no particular biological function and (iii) there is no suggestion to make different constructs in order to produce recombinant polypeptides of interest. Furthermore, there is no

indication in Menozzi that a biologically active peptide can be fused to FHA, produced in high quantities, and exhibit biological properties such as increased immunogenic properties in vivo.

The invention now provides novel compositions and methods for producing, in large quantities and high quality, recombinant polypeptides of interest, more particularly chimeric polypeptides such as chimeric antigenic polypeptides. The invention also provides novel compositions and methods to stimulate an immune response, in particular a mucosal immune response.

The invention shows that peptides of interest can be fused to FHA or particular fragments thereof, that the resulting fusion is effectively expressed in recombinant host and can be excreted outside or exposed at the surface of cells. The invention further demonstrates that the recombinant polypeptides or cells thus obtained can be used effectively to stimulate in vivo an immune response. One particular advantage of the invention is that the recombinant polypeptides or cells can be used to stimulate a mucosal immune response with high efficiency, allowing (i) the generation of high immune responses against pathogens involved in respiratory diseases, for instance, (ii) the administration of anigneic molecules by aerosols, in particular by nasal administration.

The instantly claimed compositions and methods combine several unexpected properties and advantages such as (i) highly efficient production in prokaryotic cells, in particular Bordetella bacterium, (ii) functional activity of the fused polypeptide in vitro and in vivo, (iii) facilitated purification of the recombinant polypeptide, where appropriate, (iv) where appropriate, exposure of the recombinant polypeptide at the cell surface in a biologically active conformation (v) efficient presentation of antigenic molecules to the immune system, in particular the mucosal immune system, in vitro or in vivo, etc.

None of these compositions and advantages were suggested in the applied reference which merely discloses a fundamental study of the binding properties of FHA. It is therefore believed that the invention as claimed is unobvious over the Menozzi et al. and withdrawal of the rejection is respectfully requested.

Claims 6, 8 and 9 have been rejected under 35 USC 103 as being unpatentable over Menozzi et al. and Delisse-Gathoye et al. The rejection is respectfully traversed.

It is submitted that Delisse-Gathoye et al. does not remedy the deficiencies of Menozzi et al. Indeed, as correctly pointed out by the Examiner, Delisse-Gathoye et al. reports the existence of highly homologous regions in the N-terminal domains of FHA ShlA and HpmA. However, Delisse-Gathoye et al., either alone or in combination with Menozzi et al., would not have provided the skilled artisan

with any motivation to produce secreted or anchored biologically active chimeric molecules comprising a Fha moiety. The applied references would neither have provided any reasonable expectation that such chimeric polypeptides would exhibit high immunogenic activity in vitro and in vivo and, in particular, high mucosal immunogenic activity. The observation that homology regions are present does not represent any motivation to make the instantly claimed invention which, it is submitted, is unobvious over the cited references.

Withdrawal of the rejection is therefore respectfully requested.

From the foregoing, favorable action in the form of a Notice of Allowance is respectfully requested and such action is earnestly solicited.

Respectfully submitted,

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